stimulated French endeavors for the reform of the carat by bringing it within the scope of the metric system.

The most effective worker in this direction was M. C. E. Guillaume, director of the Bureau International des Poids et Mesures at Sèvres, who urged the adoption of a carat of 200 milligrams before the International Congress in April, 1905. In January of the succeeding year, the Chambre Syndicale de la Bijouterie, Joaillerie et Orfèvrerie of Paris passed a resolution favoring the adoption of the metric carat, and in August of the same year the German federation of gem-dealers and jewelers urged its general adoption. The movement thus initiated soon spread, and by 1908 Spain had given the new carat a definite legal status, to be followed in 1909 by Japan and Switzerland. The adhesion of Italy, Bulgaria, Denmark and Norway followed in 1910, that of Holland, Portugal, Roumania and Sweden in 1911. Although it was not until 1912 that it became the legal standard in France and Germany, the law providing for its institution in the former land was passed June 22, 1909.

As in the case of all efforts to introduce metric weights or measures, the advantages of the new metric carat only very gradually became apparent in England and the United States. However, its official adoption by our Treasury Department, on July 1, 1913, as the standard for customs purposes, definitely stamps it with the seal of official acceptation here.

Belgium has already provided for the use of the new carat and England is expected to fall into line before long, so that by next year it is confidently believed there will be but one standard weight for diamonds, precious stones and pearls, the metric carat of 200 milligrams.

The paper gives a simple and easy method for converting the old carats of 205 milligrams into the new ones of 200 milligrams, and also offers many interesting details as to the history of the carat and the origin of decimal notation, the first known examples of the latter being found in a translation, published by Leonardo of Pisa in 1202, of a work by the ninth-century Arabian mathematician, Al-Khouârazmi. The first use of the decimal point is stated to occur in the arithmetic of Frances Pellos, printed at Turin in 1492.

There can be little doubt that the adoption of the metric carat in the United States will do much to favor the cause of the metric system generally in this country, as not only the thousands of jewellers but also the millions of people who buy jewelry will now learn, most of them for the first time, what a kilogram, a gram and a milligram are, when they are told that a carat equals 200 milligrams; five carats, one gram, and 5,000 carats (or 20,000 pearl grains), one kilogram.

Some additional particulars may be added from advance sheets of M. Guillaume's report to the International Conference of Weights and Measures. The Argentine Republic, Peru and Servia are all disposed to accept the new carat. In Belgium the law promulgated March 10, 1913, embraces the following article:

> In transactions concerning diamonds, pearls and precious stones, the denomination "metric carat" can be given to the weight of 200 milligrams, in derogation of articles 1 and 3 of the law of October 1, 1855.

The employment of the word "carat" to designate any other weight is prohibited.

In regard to eventual results M. Guillaume believes that the day will come when the commerce in precious stones will be confined to the employment of the ordinary metric unities; the establishment of the carat as a fiftieth part of a grain will then have constituted a stage in this definite reform, and one greatly favoring it.

George F. Kunz

SPECIAL ARTICLES

THE MECHANISM OF FERTILIZATION

In previous papers[1] I have described the secretion of a substance by the ova of the sea-

[1] Science, N. S., Vol. 36, pp. 527–530, October, 1912, and *Journ. Exp. Zool.*, Vol. 14, No. 4, pp. 515–574, May, 1913.

urchin, *Arbacia,* in sea water, which causes agglutination of the sperm of the same species. The eggs of *Nereis* also secrete a substance having a similar effect upon its sperm. I therefore named these substances sperm-isoagglutinins. During the present summer I have ascertained that in the case of *Arbacia,* and presumably also of *Nereis,* the agglutinating substance is a necessary link in the fertilization process and that it acts in the manner of an amboceptor, having one side-chain for certain receptors in the sperm and another for certain receptors in the egg. As this substance represents, presumably, a new class of substances, analogous in some respects to cytolysins, and as the term agglutinin defines only its action on sperm suspensions, I have decided to name it fertilizin.

My main purpose this summer was to study the rôle of the *Arbacia* fertilizin in the fertilization of the ovum.

1. *The Spermophile Side-chain.*—The first need in such a study was to develop a quantitative method of investigation, and this was done for *Arbacia* as follows: The agglutinative reaction of the sperm in the presence of this substance is, as noted in previous studies, reversible, and the intensity and duration of the reaction is a factor of concentration of the substance. The entire reaction is so characteristic that it was possible to arrive at a unit by noting the dilution at which the least unmistakable reaction was given. This was fixed at about a five- or six-second reaction, which is counted from the time that agglutination becomes visible under a magnification of about 40 diameters until its complete reversal. The unit is so chosen that a half dilution gives no agglutination of a fresh 1 per cent. sperm suspension. It was then found that the filtrate from a suspension of 1 part eggs left for ten minutes in 2 or 3 parts sea water would stand a dilution of from 800 to 6,400 times, depending on the proportion of ripe eggs and their condition, and still give the unit reaction. Such solutions may then be rated as 800 to 6,400 agglutinating power, and it is possible, therefore, to determine the strength of any given solution. This gives us a means of determining the rate at which eggs are producing fertilizin in sea water.

Determinations with this end in view showed that the production of fertilizin by unfertilized eggs of *Arbacia* in sea water goes on for about three days and that the quantity produced as measured by dilution tests diminishes very slowly. Such tests are made by suspending a given quantity of eggs in a measured amount of sea water in a graduated tube; the eggs are then allowed to settle and the supernatant fluid poured off and kept for testing. The same amount of fresh sea water is then added and the eggs stirred up in it, allowed to settle, the supernatant fluid poured off for testing, and so on. In one series running three days in which the quantity of eggs was originally 2 c.c. and the total volume of sea water and eggs in the tube 10 c.c., 6 to 8 c.c. being poured off at each settling, thirty-four changes were made and the agglutinating strength of the supernatant fluid diminished from 100 at first to 20 at the end. Simultaneously, with this loss of agglutinating strength, two things happen: (1) the jelly surrounding the eggs undergoes a gradual solution; (2) the power of being fertilized is gradually lost.

It is obvious that the presence of fertilizin in such considerable quantities in so long a series of washings shows either (1) that solution of the jelly liberates fertilizin, or else (2) that the eggs secrete more fertilizin each time they are washed. Both factors enter into the case inasmuch as (1) eggs killed by heat (60° C.) will stand 14 or 15 such washings, but with more rapid decline of agglutinating power than the living eggs. The jelly is gradually dissolved away in this case also, and is presumably the only possible source of the agglutinating substance. (2) Eggs deprived of jelly by shaking continue to produce the fertilizin as long as eggs with jelly, though in smaller quantities at first, and they are equally capable of fertilization.

The fertilizin is therefore present in large quantities in the jelly, which is indeed saturated with the substance, but the eggs continue to produce it as long as they remain alive and unfertilized. When the eggs are

fertilized the production of this substance suddenly ceases absolutely.

The total disappearance of fertilizin from fertilized eggs can not be demonstrated unless the fertilizin-saturated jelly with which the eggs are surrounded be first removed. This is very easily done after membrane formation by six vigorous shakes of the eggs in a half-filled test tube. Three or four washings then are sufficient to remove the remains of the jelly, and the naked eggs no longer produce the substance.

Such disappearance may be due either to complete discharge from the egg, or to fixation of all that remains by union with some substance contained in the egg itself. That such a substance—anti-fertilizin—exists in the egg can be shown by a simple test-tube experiment: If eggs deprived of jelly are washed 34 times in sea water during three days, they are so exhausted that they produce but little fertilizin; the supernatant fluid may be charged only to the extent of 2 to 10 units. The eggs are now on the point of breaking up. If they are then vigorously shaken and broken up so that the fluid becomes colored with the red pigment of the eggs, it will be found that agglutinating power has entirely disappeared from the solution. The fertilizin present has been neutralized. The same phenomenon may be demonstrated also by treating eggs, deprived of jelly in order to get rid of excess of fertilizin, with distilled water which lakes the eggs and extracts the anti-fertilizin.

It is probable, therefore, that any excess of fertilizin remaining in the egg not bound to the sperm is neutralized by this combination, and polyspermy is thereby prevented.

We have noted (1) the secretion by unfertilized eggs in sea water of a sperm agglutinating substance, fertilizin; (2) the extreme avidity of the sperm for it as shown by dilution tests; (3) in my previous papers the fixation of this substance in sperm-suspensions of the same species (quantitative measurements will be given in the complete paper); (4) the sudden cessation of fertilizin production by fertilized eggs; (5) the existence of an anti-fertilizin in the egg; (6) in eggs submitted to a series of washings decrease of the fertilization capacity with reduction of the fertilizin. The fact that fertilized eggs can not be refertilized is associated with the absence of free fertilizin in them; (7) I may add that, similarly, eggs in which membrane formation has been induced by butyric acid can not be fertilized by sperm and they contain no free fertilizin.

It is therefore very probable that the substance in question is essential for fertilization.

It may be maintained that these facts do not constitute demonstrative evidence of the necessity of this substance for fertilization, for the presence or absence or diminution of this material associated with presence or absence or decrease of fertilizing power could always be regarded as a secondary phenomenon. However, the second part of this paper dealing with the other, or ovophile side-chain of the fertilizin, strongly reinforces the argument.

Before passing on to this, I may be allowed to note some other properties of the fertilizin: In my previous papers I noted the extreme heat-resistance of the fertilizin, being only slowly destroyed at 95° C. I also noted that strongly agglutinating solutions of *Arbacia* may contain a substance which agglutinates *Nereis* sperm and stated that this was probably different from the iso-agglutinating substance. This turns out to be the case and the two can be readily separated. The substance must possess great molecular size, as it is incapable of passing through a Berkefeld filter. It is also non-dialyzable; it does not give the usual protein reactions, a fact for the determination of which I am indebted to Dr. Otto Glaser.

2. *The Ovophile Side-chain.*—Assuming, then, that the union of this substance with the spermatozoon enters in some significant way into the process of fertilization, the problem was to ascertain in what way. The simplest idea, viz., that the union is in itself the fertilization process, was soon shown to be untenable, for the reason that the perivisceral

fluid (blood) of the sea-urchin, especially of ripe males and females, often contains a substance which absolutely inhibits fertilization in the presence of any quantity of sperm, but that this substance has no inhibiting effect at all upon the sperm-agglutination reaction. It does not enter into combination with the spermophile side-chain. In other words, the binding of the agglutinin by the sperm may be complete, but in the presence of an inhibitor contained in the blood none of the usual effects of insemination, no matter how heavy, follow.

The details of the experiments upon which the above statement depends are too complex for consideration here. But they showed that the effect is neither upon the egg alone nor upon the sperm alone, for both may stand for some time in the presence of this agent and after washing be capable of normal behavior in fertilization, though there may be some decrease in the percentages. No poisonous effect is involved on either sexual element.

The next suggestion was fairly obvious, viz., that the substance which we had been calling agglutinin, on account of its effect upon the spermatozoa, is in reality an amboceptor with spermophile and ovophile side-chains, and that the binding of the sperm activates the ovophile side-chains which then seize upon egg receptors and fertilize the egg. If this were so, it is obvious that the spermatozoon is only secondarily a fertilizing agent, in the sense of initiating development, and that the egg is in reality self-fertilizing, an idea which agrees very well with the facts of parthenogenesis and the amazing multiplicity of means by which parthenogenesis may be effected. For the agents need only remove obstacles to the union of the amboceptor and egg receptor.

The inhibiting action of the blood from this point of view is a deviation effect due to occupancy of the ovophile side-chain of the amboceptor, either because the inhibitor in the blood is an anti-body to the amboceptor or because it possesses the same combining group as the egg receptor. In such a case, the ovophile group of the amboceptor, being already occupied by the inhibitor, fertilization could not take place.

Fortunately, this idea is susceptible of a ready test; for, if the blood acts in this way in inhibiting fertilization, all that is necessary to neutralize the inhibiting action would be to occupy the inhibitor by the amboceptor (fertilizin) for which *ex. hyp.* it has strong affinity. This experiment was repeated many times in different ways with various dilutions, and the result was always to lessen or completely remove the inhibiting action of the blood.

The plan of such an experiment is this: to divide the filtered blood (plasma) in two parts, one of which is used for control while the other is saturated with fertilizin by addition of eggs. In ten minutes the latter are precipitated by the centrifuge and the supernatant fluid filtered. Fertilizations are then made in graded dilutions of this and the control blood. In some cases the inhibiting action of the blood was completely neutralized, and in all largely neutralized.

The results so far are in agreement with the theory. But if it be true that the egg contains its own fertilizing substance, it might also be possible to induce parthenogenesis by increasing the concentration of this substance to a certain point; though it is conceivable that no increase in concentration would break down the resistance that normally exists to union of the amboceptor and egg receptors. As a matter of fact, Dr. Otto Glaser[2] has shown this summer that a certain amount of parthenogenetic action may be induced in *Arbacia* in this way. I have been in consultation with Dr. Glaser during part of his work and can confirm his statements.

In connection with the assumption that the sperm activates an already existing side-chain of a substance contained in the egg itself, I may be allowed to cite the following statement of Ehrlich:

> The significance of the variations in affinity will be discussed connectedly at a subsequent time. We shall content ourselves here by pointing out

[2] SCIENCE, N. S., Vol. XXXVIII., No. 978, September 26, 1913, p. 446.

that an understanding of the phenomena of immunity is impossible without the assumption that certain haptophore groups become increased or decreased in their chemical energy, owing to changes in the total molecule. Chemically, such an assumption is a matter of course.[3]

This principle might explain the activation of the fertilizing amboceptor by the sperm.

The question will of course be raised whether there is not another and simpler interpretation of the facts. There are three general classes of these facts: (1) the sperm agglutination phenomena, and the apparent necessity of the agglutinating substance for fertilization; (2) the presence of an inhibiting agent in the blood, especially of ripe males and females; (3) the neutralization of this inhibiting agent by the agglutinating agent (amboceptor). It may be questioned whether these facts have the particular causal nexus that I have given them. But I think it would be difficult to construct a theory taking account of all the facts which would differ essentially from that presented here.

The theory is really extremely simple in its character, and the facts on which it rests are readily tested. It has proven a most valuable working hypothesis; indeed, many of the facts referred to were discovered only after the theory was formed. It has the advantage of offering one theory for initiation of development whether by fertilization or by parthenogenesis. It is capable of explaining the whole range of specificities in fertilization by assuming a specific fertilizin for each species. It furnishes the foundation for the chemical conceptions necessary to any theory of fertilization, and it is susceptible of experimental test.

It will be seen that inhibition of fertilization may occur by block in any part of the mechanism.

1. Through loss of fertilizin by the egg.
2. Through occupancy of the sperm receptors.
3. Through occupancy of the egg receptors.
4. Through occupancy of the ovophile side-chain of the amboceptor (fertilizin).
5. Through occupancy of the spermophile side-chain group.

Of these I have shown the occurrence of the first, fourth and fifth in *Arbacia*. The first in the case of long-washed eggs; the fourth in the case of the inhibitor contained in the blood; the fifth is, I believe, the mechanism for prevention of polyspermy.

The mechanism of fertilization appears to be the same in *Nereis*, though I have not a complete set of data. However, the data that I have are in accord with the theory, and will be described in the complete paper.

I should perhaps state specifically that the location of the fertilizin is in the cortex of the egg.

It seems to me probable that the activation of the fertilizin is by no means confined to that bound by the single penetrating sperm, but that activation once set up spreads around the cortex. The supernumerary spermatozoa that fail to enter the egg may also play a part by setting up centers of activation. In this connection Glaser's contention that several spermatozoa at least are necessary for fertilization is of great interest. The nature of the effect of the activated fertilizin on the egg is analogous in some respects to a superficial cytolysis, in this respect agreeing with Loeb's theory. But the "lysin" is contained in the egg, not in the sperm, as Loeb thought; if cytolysis is involved, it is a case of autocytolysis. This may involve increase of permeability, the effects of which R. S. Lillie has especially studied. I mention these possibilities in order to point out that the conception contained in this paper is not in conflict with the well-established work of others.

In conclusion, I may point out that the theory assumes a form of linkage of sperm and egg components by means of an intermediate body that may find a place in the study of heredity. The detailed experiments will be published later.

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September 1, 1913

[3] "Collected Studies in Immunity," p. 220.